HUMAN PHOTOREACTIVATING ENZYME

ACTION SPECTRUM AND SAFELIGHT CONDITIONS

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ABSTRACT The action spectrum for photoreactivation by enzymes from human leukocytes and fibroblasts extends from 300 to approximately 600 nm with a maximum near 400 nm. The ability of the human enzymes to utilize light of wavelengths greater than 500 nm suggested that yellow or gold lights conventionally used as safelights for photoreactivation might serve as sources of photoreactivating light for these enzymes. Experiments using lights with a range of spectral outputs confirm that the standard yellow "safe" lights do produce photoreactivation by the human but not the *Escherichia coli* enzyme.

INTRODUCTION

The photoreactivating enzyme repairs damage produced in DNA by ultraviolet light (220–300 nm) by monomerizing cyclobutyl pyrimidine dimers in a reaction requiring longer wavelength ultraviolet or visible light (>300 nm). Although photoreactivating enzymes had been found in all phylogenetic groups with the singular exception of the placental mammals (Cook and McGrath, 1967), recent data indicate that these mammals also contain the enzyme (Sutherland, 1974*b*; Sutherland et al., 1974; Harm, 1974). Why was photoreactivation in mammalian cells so difficult to detect? Our results reveal an unusual property of human photoreactivating enzymes which may have interfered with its detection in vivo and in vitro:[1] the human enzymes utilize light of longer wavelengths than do the extensively studied enzymes from yeast (Setlow, 1966, and Harm and Rupert, 1970), *E. coli* (Jagger et al., 1970), and *Streptomyces griseus* (Jagger et al. 1969). In particular, the human enzyme can utilize yellow light from lamps commonly used as "safelights" in photoreactivation experiments. Our results indicate that only red lights which emit no wavelengths shorter than 600 nm should be considered for use as safelights for photoreactivation by the human enzyme.

[1]Other factors which may have impeded the detection of the human enzyme have been discussed elsewhere (Sutherland, 1974*a*, *b*).

METHODS AND MATERIALS

Photoreactivating Enzyme

Human leukocyte enzyme was prepared according to the method of Sutherland (1974*a*): freshly drawn, heparin-treated blood (30 cm^3) was treated with 10 cm^3 of Plasmagel (Roger Bellon Laboratory, Nevilly, France). The leukocyte fraction was centrifuged at 500 *g* for 5 min, washed with 0.15 M NaCl, and sonicated in 0.01 M tris, pH 7, 0.1 mM dithiotheritol, 0.1 mM ethylenediaminetetraacetate (Buffer E). The enzyme was purified by ammonium sulfate precipitation (60% saturation) and isoelectric focusing in a 140 ml glycerol gradient containing pH 3–10 LKB ampholytes.

Human fibroblasts were grown in Dulbecco's modified minimal essential medium (Eagle, 1959). Cells were washed with 0.15 M NaCl, scraped into the same solution, washed by centrifugation as above, suspended in Buffer E and sonicated in a Kontes sonicator (Kontes Glass Co., Vineland, N. J.).

Enzyme Assays

Photoreactivating enzyme activity was determined as previously described (Sutherland and Chamberlin, 1973). In brief, cell extracts or purified enzyme was added to 0.2 ml of a solution containing 0.02 M sodium phosphate buffer, pH 7.2, 0.1 mM dithiotheritol, and 0.1 mM ethylenediaminetetraacetate, and 30–100 pmol of ^{32}P-labeled, purified T7 DNA which had been exposed to 300 J/m^2 of ultraviolet radiation from a 15 W germicidal bulb. One sample was kept in the dark, while duplicates were exposed to photoreactivating light (see below). The dimer content of the samples was then determined as follows: Samples were digested to a mixture of inorganic phosphate, mononucleosides, and dimer-containing oligonucleotides (Setlow et al., 1964) by the addition of 10 μg DNase I (5 min, 37°), 10 μl of 1 M tris pH 8, plus 100 μg of venom phosphodiesterase, and 1 μg of bacterial alkaline phosphatase (60 min, 37°). The reaction was terminated by the addition of 10 μl of 0.46 N HCl; mononucleosides and dimer-containing oligonucleotides were separated from $^{32}P_i$ by absorption to Norit (L. A. Salomon & Bros., Inc., Port Washington, N. Y.), filtering and washing with 0.1 M sodium pyrophosphate-0.1 M sodium phosphate, pH 6. Samples were counted in a Nuclear-Chicago planchet counter (Nuclear-Chicago, Des Plaines, Ill.). Enzyme activity was calculated from the difference between the radioactivity on Norit of a sample exposed to photoreactivating light from that resulting from the sample kept in the dark. Units of enzyme activity are picomoles per milligram per hour. Protein concentrations were determined by the Lowry method (1951).

Samples were prepared in a cabinet illuminated by a 25 W red incandescent bulb; the light was extinguished during digestion.

Fluorescent Lamps

Sample tubes in a 37° water bath were exposed for 30 min to light from 40 W Westinghouse Warm White or Sylvania Lifeline F 40 G0 yellow fluorescent bulbs located 12 in above the samples.

Incandescent Lamps

Sample tubes in a 37° water bath were exposed for 15 min to light from a blue, yellow, or red 25 W Westinghouse "Sign Service Group" incandescent bulb with transparent colored envelopes or 60 W Sylvania Soft White white or Bug Foiler yellow bulbs with opal envelopes. The bulbs were mounted in an aluminum reflector 6 in above the sample. The irradiation was carried out in the cabinet described above.

Relative spectral distributions of the incandescent lamps were measured with a multi-purpose

spectrometer which will be described elsewhere. Light from the bulb passed through a rotating sector "chopper" (PAR Inc.) and a double grating monochromator (model 1672; Spex Industries, Inc., Metuchen, N. J.) and onto an EMI 9558QB photomultiplier (EMI Gencom Div., Plainview, N. Y.). The amplitude modulated phototube current was amplified and detected by a PAR model 124 phase sensitive detector equipped with a PAR model 184 preamplifier and displayed on an *x-y* recorder. The emission spectra measured with this apparatus were not corrected for the wavelength dependence of the detection system. The sensitivity of the photomultiplier decreases at longer wavelengths reaching zero at about 950 nm. These spectra do indicate the relative intensities of different bulbs at a given wavelength and the shortest wavelength produced by each bulb.

Action Spectra

Light for photoreactivation was supplied by a 1,000 W high pressure air-cooled mercury arc in a Schoeffel Model LH 151 N housing (Schoeffel Instrument Corp., Westwood, N. J.), energized by a Christie Model SCX1200-12S current regulated power supply (Christie Electric Corp., Los Angeles, Calif.). Emission lines were isolated by interference filters (Detric Optical Co., Marlboro, Mass.) or a 200 mm focal length monochromator with a concave holographic grating (J-Y Optical Systems, Metuchen, N. J.). A 75 mm filter containing water or copper sulfate solution removed infrared radiation from the beam. A 1.5-in diameter quartz lens (focal length 100 mm) formed an image of the monochromator grating on the sample. A first surface mirror mounted at 45° deflected the horizontal beam so that it passed vertically down the axis of the sample tube (maintained at 37° in a circulating water bath) to prevent distortion of the spatial intensity distribution of the beam by focusing effects.

The mirror could be rotated out of the beam which then fell on the detector element of a Hewlett-Packard radiant flux meter (model 8330 A; Hewlett-Packard Co., Palo Alto, Calif.) located the same distance from the mirror as the sample. The intensity of the irradiating beam was adjusted by varying the power supplied to the arc, interposing neutral density filters or adjusting the slit width and wavelength setting of the monochromator. The fluence at 313 nm was 0.26 mW/cm^2; at longer wavelengths this was decreased by a factor of $313/\lambda$ so that a constant number of photons reached the sample per unit time. All samples in one experiment were irradiated for the same time (usually 20 min). Preliminary experiments showed that these fluences were light limiting and hence relative photoreactivation could be taken to be proportional to the measured enzyme activity.

RESULTS AND DISCUSSION

Action Spectra

The action spectra of human fibroblast and leukocyte photoreactivating enzymes (PRE) extends from 300 to about 600 nm with maxima near 400 nm (Fig. 1). In contrast, the action spectrum of *E. coli* PRE extends from 300 to about 500 nm with a maximum near 380 nm (Jagger et al., 1970). Comparison of these spectra shows (*a*) PRE from fibroblasts and leukocytes have similar or identical action spectra, (*b*) the spectrum of the human enzyme peaks at a longer wavelength than does the *E. coli* PRE, and (*c*) the spectrum of the human enzyme extends to longer wavelengths than that of the *E. coli* enzyme.

Determination of Safelight Conditions

Since the action spectra of photoreactivating enzyme from *E. coli* and yeast do not extend past 500 nm (Jagger et al., 1970; Setlow, 1966; and Harm and Rupert, 1970)

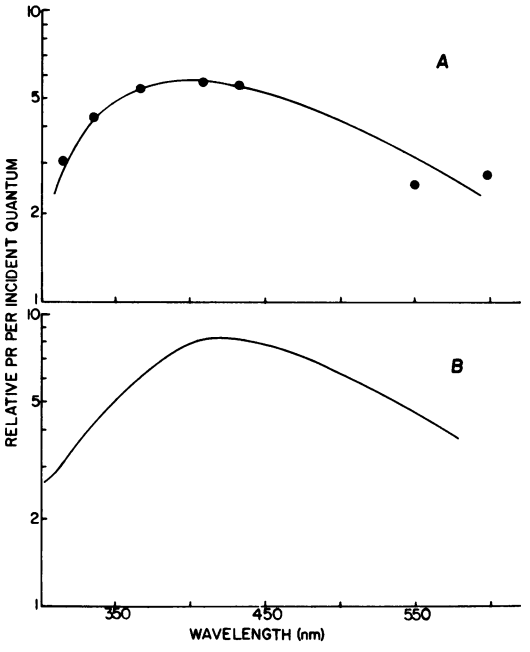

FIGURE 1 Action spectra for photoreactivation for enzyme from (*A*) human fibroblasts and (*B*) human leukocytes (Sutherland et al., 1974).

yellow lamps (e.g. Sylvania Bug Foiler or General Electric Gold) can serve as safelights for experimental manipulations. However, the action spectra shown in Fig. 1 suggested that yellow lights might not be safelights for the human enzyme. We thus measured photoreactivation produced by human and *E. coli* PRE in the presence of light from a yellow incandescent bulb. Table I, line 2 shows that the yellow light can produce photoreactivation by the human but not by the *E. coli* enzyme. White light from an otherwise similar bulb produced photoreactivation by both enzymes (Table I, line 1), thus indicating this is indeed a wavelength-dependent difference. The spectral

TABLE I

NET PHOTOREACTIVATING ENZYME ACTIVITY (%) OF HUMAN (HESM) AND *E. COLI* ENZYMES FOR FIVE INCANDESCENT LAMPS

	Lamp*	HESM	*E. coli*
1	60 W white	100	100
2	60 W yellow	83	3
3	25 W blue	100	100
4	25 W yellow	70	0
5	25 W red	5	0

*Spectral "outputs" shown in Fig. 2.

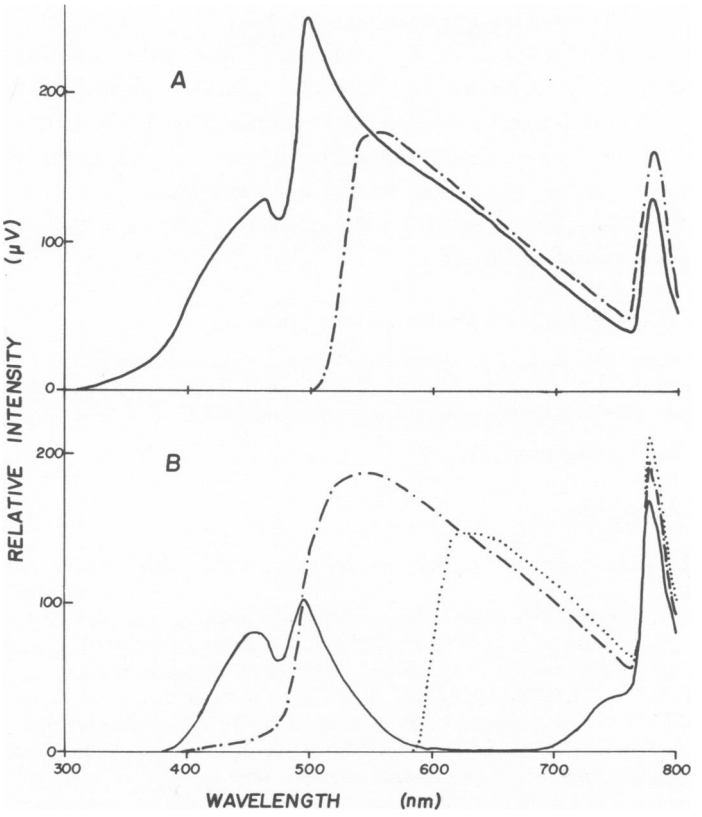

FIGURE 2 The emission spectra of (*A*) 60 W white (—) and yellow (— · —) frosted and (*B*) 25 W blue (——), yellow (—·—), and red (·····) clear incandescent bulbs. Data are uncorrected for wavelength-dependent sensitivity of the detection system which causes an apparent decrease in output at longer wavelengths and the structure between 450 and 500 nm in the spectra of the blue and white bulbs.

outputs of these bulbs are shown in Fig. 2 *A*. Similar results were obtained with white and yellow fluorescent lamps. Thus, yellow lights are not safelights for the human photoreactivating enzyme.

The long wavelength limit of the action spectrum of the human enzyme is not determined precisely by the data in Fig. 1. We thus tested the ability of a red incandescent bulb to produce photoreactivation by the human enzyme. The relative spectral output of the red bulb (and also of blue and yellow bulbs of similar types which served as controls) is shown in Fig. 2 *B*. The emission of the red bulb is principally above 600 nm with no detectable output below 585 nm. Table I, lines 3, 4, and 5 show that for the human enzyme the amount of photoreactivation produced by the red bulb is much less than that from the blue or yellow bulbs. Neither the red nor yellow bulbs produced significant photoreactivation by the *E. coli* enzyme, although the blue light did serve as a good source of photoreactivating light. These data indicate that *only* red lamps

which contain no wavelengths less than 600 nm should be considered for use as safelights for the human enzyme. The desirability of a long wavelength emission cutoff must be balanced by the requirement for sufficient light for experimental manipulation. In any case, all samples should receive the same (minimal) exposure to the safelight.

The ability of the human photoreactivating enzyme to utilize yellow light as an energy source may have contributed to the apparent lack of photoreactivation in human cells and the apparent lack of photoreactivating enzyme activity in human cell extracts. (See Sutherland, 1974 *a*, *b*.)

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